Results from preliminary screening of approved fungicides for efficacy against Hymenoscyphus fraxineus (Chalara fraxinea), the cause of ash dieback

Defra has funded a three year research project (2013-2016) to develop strategies to mitigate the spread of ash dieback in the UK. In October 2013, details of the laboratory and field work being undertaken to test efficacy of currently available fungicides against ash dieback was published on the Defra website. All 14 products (plus an additional three tested as a result of information from the manufacturer) were tested in laboratory experiments to measure the effectiveness of the fungicides against mycelial growth in broth culture. Results from the laboratory testing are shown in Table 1 and are expressed as an EC_{50} : the effective concentration in ppm causing 50% inhibition of growth compared to the control.

Table 1. Efficacy of fungicides against mycelial growth

	Active Ingredient	Product	Fungicide Group	EC ₅₀ (ppm)
1	Myclobutanil	Systhane 20EW	Triazole	*
2	Cyproconazole	Alto 100 SL	Triazole	***
3	Prothioconazole	Proline	Triazole	***
4	Fenbuconazole	Indar 5EW	Triazole	***
5	Flutriafol	Consul	Triazole	-
6	Azoxystrobin	Amistar	Strobilurin	***
7	Fluxapyroxad	Imtrex	SDHI#	***
8	Bixafen/prothioconazole	Aviator 235 XPro	SDHI/triazole	***
9	Boscalid/pyraclostrobin	Signum	SDHI/strobilurin	***
10	Mancozeb	Cleancrop Mancozeb	Dithiocarbamate	**
11	Pyrimethanil	Scala	Anilinopyrimidine	*
12	Dithianon	Dithianon WG	Quinone	***
13	Garlic extract (allicin)	Eagle Greencare	Organosulfur	***
14	Copper oxychloride	Cuprokylt FL	Inorganic copper	*
15	Cyproconazole + trifloxystrobin	Escolta	Triazole/strobilurin	***
16	Trifloxystrobin + tebuconazole	Nativo 75 WG	Strobilurin/triazole	***
17	Tebuconazole	Folicur	Triazole	***

^{# =} Succinate dehydrogenase inhibitor

^{*** =} EC_{50} value <1 ppm (high activity)

^{** =} EC₅₀ value between 1 and 10 ppm (moderate activity)

^{* =} EC₅₀ value between 10 and 100 ppm (low activity)

^{- =} product failed to work in the test; no result available until further work is done this season

The EC $_{50}$ values show that individual products containing chemistry from the triazole, strobilurin, SDHI, quinone or organosulphur groups had significant efficacy against mycelial growth of *C. fraxinea* (products with an EC $_{50}$ of less than 1 ppm were considered highly effective). The range of fungicide groups shown to have significant efficacy is important for the development of an effective fungicide control strategy, particularly as *H. fraxineus* is an actinomycete fungus and therefore has a very high risk of developing fungicide resistance. The risk of resistance developing will be most effectively managed by use of several fungicide groups within a control strategy, preferably as a formulated mixture.

Field trials were established at two locations within an outbreak site in East Yorkshire. At the first site (field margin) 15 products were tested (products 3 and 8 were omitted in favour of the additional three products (15-17)). The second site (woodland) was limited in space so only 5 products were tested (2, 9, 15, 16 and 17). Once the pathogen was known to be actively sporulating (July 2013) ash saplings (approx. 2-3 years old) were given an initial spray (20 replicates per treatment), set up at the outbreak site and then sprayed every two weeks until early October (seven sprays total). This very intensive spray programme was used to measure the maximum control which could be achieved from the treatments. Visual assessments of foliar disease symptoms were made every week. First symptoms appeared in mid-September and full disease assessments of the trial were made on 30th September and 7th October, with symptoms confirmed by PCR. Two final assessments were made in the following spring/early summer (23rd May & 23rd June 2014) when the saplings were scored for the presence of stem lesions, which were later confirmed by PCR. Due to the prolonged hot dry weather the trial at the more exposed field margin site did not survive until the end of the experiment. Although not all products are represented, the surviving subset of the trials has delivered results for some of the key products/fungicide groups which were indicated to be highly effective in the laboratory tests.

Visual assessments of foliar disease on 7th October showed that 85% of the untreated trees had developed leaf symptoms of ash dieback (Figure 1). All the treatments tested at woodland site reduced disease levels compared to the control with Nativo, Signum and Escolta being the most effective. Results on the average number of leaves showing symptoms (Figure 2) confirmed the effectiveness of these three treatments with Nativo and Signum reducing foliar symptoms to less than 5% of leaves affected compared to 44% in the controls. The lesion assessment carried out in spring/summer 2014, identified the trees in which the pathogen had successfully infected the tree, rather than being shed solely as a leaf infection during the autumn. These results indicate that the fungicide treatments completely prevented progression of the foliar disease into the stems of the saplings whereas 60% of the controls had stem symptoms (Figure 3), with lesions measuring an average of 60cm in length.

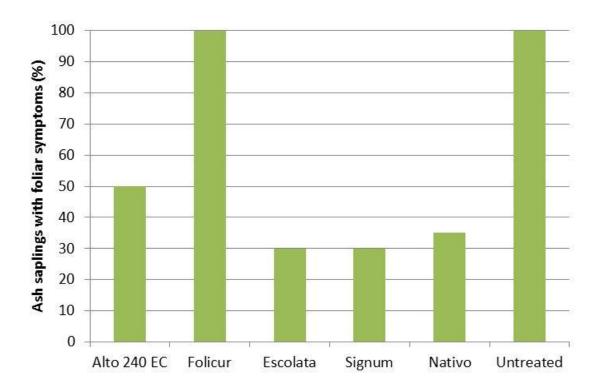


Figure 1. Percentage ash saplings affected by foliar symptoms following fungicide treatments (assessed 7/10/2013).

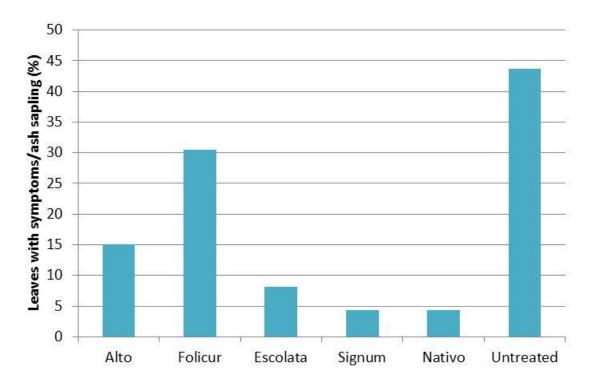


Figure 2. Percentage leaves affected by foliar symptoms following fungicide treatments (assessed 7/10/2013).

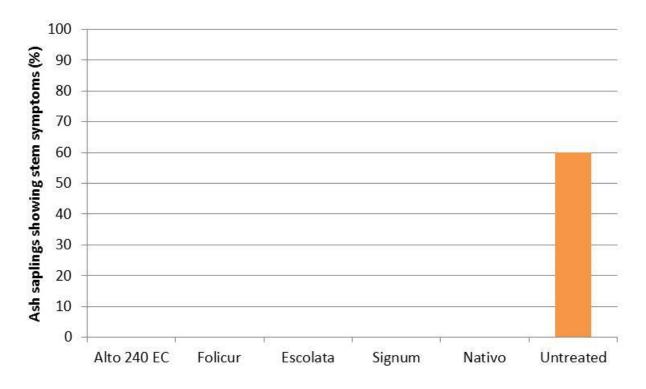


Figure 3. Percentage ash saplings affected by stem lesions following fungicide treatments (assessed 23/06/2014)

Conclusions

- There are a number of products representing a range of fungicide groups which show strong activity against *C. fraxinea* in the laboratory
- Field trials on a limited number of products show that these chemicals can be used to significantly reduce both foliar and stem symptoms in young trees
- Results show that products may not prevent leaf infection but can prevent progression of the infection into the stem
- The levels of disease control in the trials were only achieved through use of a spray
 programme involving seven sprays applied at two-weekly intervals through the risk period
 for infection
- Use of such an intensive spray programme is both impractical and unsustainable in the natural environment.
- Control strategies for ash dieback involving foliar applications of fungicides are likely to require multiple spray applications and will only be appropriate for protection of young saplings, eradication of very early disease outbreaks or short term protection of high value specimens

Next steps

- Laboratory tests to determine efficacy of products on ascospore germination
- Field trials set up in summer 2014 to test optimal timing (early, mid, late season) and frequency of sprays (two, four or six sprays) for disease management
- Stem injection trials on young saplings using three of the effective products